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13. ABSTRACT (Maximum 200 Words)
Bone fluid flow is hypothesized to initiate aberrant remodeling which can ultimately compromise bone quantity and quality. Thus, pathologic response of load-induced fluid flow can potentially damage tissue viability, and initiate bone's remodeling process, ultimately leading to the stress fracture syndrome. The research goal is evaluated through two primary specific aims: (1) repetitive fluid flow, as dependent on magnitude and duration, will stimulate pathological remodeling; and (2) cyclic intramedullary pressure (ImP) will induce nutrient vessel remodeling and constriction, and thus partially reduce nutritional flow to the cortex.
Results have shown that within the physiological range, bone formation is proportional to applied fluid flow stimulation. While the pressure exceeds the physiological intensity of falls within the pathologic range, i.e., addition to normal physical loading, it triggers extensive remodeling and thus even weaken the quality of bone, i.e., increase intracortical porosity and induce lesions. Repetitive cyclic fluid flow in bone has shown an effect on the nutrient vessel remodeling inducing vessel wall thickening, which may potentially contribute to pathological remodeling in bone. These results may provide insight of fluid flow in physiological and pathological remodeling and its role in stress fracture.

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A. Introduction

Musculoskeletal complications that arise in the training of recruits represent a critical health problem for the military. Stress fractures almost exclusively occur in physically active individuals, e.g., dancers, joggers, and soldiers, in a variety of skeletal location but mostly in lower limb, e.g., femur, tibia, and calcaneus [2]. The impact of stress fractures is severe, which 70% of all stress fractures are reported in runners and is ranked as the most common risk to running [4].

It is widely accepted that stress fractures are the result of accumulated fatigue microdamage, yet there several other candidate mechanical factors could – as likely - contribute the deterioration of the bone tissue. For example, load-induced interstitial fluid flow, a primary regulator of bone adaptive responses, could initiate the stress fracture syndrome by upregulating the resorption of the cortex, independent of a repair process catalyzed by material corruption. Indeed, preliminary data from our lab has shown that intracortical fluid flow can be induced not only by bone matrix strain, but by the intramedullary pressure (ImP) generated during loading. Increased ImP pressures arising from redundant axial loading in turn severely compromises perfusion of the bone tissue, and thus alters the vascular fluid supply. Thus, pathologic levels of load-induced flow can damage tissue viability, and thus initiate bone's remodeling process, ultimately leading to the stress fracture syndrome. Importantly, alterations in ImP fluid pressure can stimulate remodeling even under conditions of minimal bone strain, and thus pathology can arise even in the absence of intense physical loading of the bone tissue. In the work proposed, we hypothesize that:

Persistent levels of bone fluid flow, physiologic in magnitude, will initiate aberrant remodeling which ultimately compromises bone quantity and quality.

Thus, the goal of this research is to evaluate 1) repetitive fluid flow, as dependent on magnitude and duration, will stimulate pathological remodeling and ultimately compromise the material properties of the bone; and 2) cyclic intramedullary pressure can alter the nutrient vessel blood supply and partially reduce nutritional flow to the cortex.

The stress fracture is considered as an ongoing repair due to bone remodeling, stimulated by the gradual accumulation and growth of microcracks within the matrix. It is proposed that mechanical loading results in deformation of bone matrix and the substantial interstitial fluid space, which generates pressure gradients and further induces interstitial fluid flow [6]. Cortical bone is composed of a solid matrix phase and an interstitial fluid phase [7]. Approximately 80%~85% of bone volume consists of solid matrix, such as mineral and collagen, while the rest consists of fluid phase such as interstitial fluid in the porous medium [1,5,9,8,11]. This fluid fills in the various spaces and channels in bone [7,11]. The motion of pore fluid under mechanical loading may play an important role in the mechanism of bone cell sensing, signaling and responding to physical stimuli, as well as nutrient transport. This intracortical fluid flow is considered a critical mediator of bone mass and morphology [3,5,9,8,10]. Fluid flow may represent a critical role to explain strain magnitude, strain rate and gradient regulated bone formation, remodeling, and weakening of bone.

The mechanisms of fluid flow in bone may be engaged with physical stimulation genearated remodeling through repetitive oscillatory fluid pressure, fluid shear stress on the cellular activities, and altering nutrient supply and waste removal of bone. Fluid flow as a pathogenic factor contributing to aberrant remodeling may depend on the magnitude of stimulus, i.e., hydraulic fatigue, and triggered by alteration of nutrients supply. Our results may provide promising evidences that bone fluid flow is likely an etiology factor initiating bone remodeling and substantial stress related fractures.

B. Research accomplishments

In this study, we hypothesize that persistent levels of bone fluid flow, physiologic in magnitude, can initiate aberrant remodeling which can ultimately compromise bone quantity and quality. Thus, pathologic response of load-induced fluid flow can potentially damage tissue viability, and initiate bone's remodeling process, ultimately leading to the stress fracture syndrome. The PI and the research team are grateful for the opportunity of this research grant from the USAMRMC. The preliminary results from this research may lead to a new insight in understanding fluid flow induced bone remodeling and its resultant stress fracture mechanism. The research goal was initially proposed to be achieved through two primary sub-hypotheses and specific aims: (1) repetitive fluid flow, as dependent on magnitude and duration, will stimulate pathological remodeling *in the absence of matrix strain* and ultimately compromise the material properties of the bone; and (2) cyclic intramedullary pressure (ImP) will cause nutrient vessel remodeling and constriction, and thus partially reduce nutritional flow to the cortex.

Results of the study demonstrate progressive achievements in the areas of (a) dose dependence of bone remodeling elucidated by dynamic fluid flow stimulation in a disuse model; (b) trabecular bone adaptation induced by low intensity, high cycle number oscillatory intramedullary pressure stimulation in an intact model; (c) remodeling initiated by repetitive fluid flow serves as an etiologic factor for osteopenic lesions and stress fractures in an intact model; (d) long duration, repetitive, oscillatory fluid loading initiates the reduction of bone blood volume flow in nutrient vessels; and (e) repetitive, long duration, intramedullary fluid pressure oscillatory loading can generate nutrient vessel wall remodeling and potentially reduce blood flow in bone

(1) Dose dependence of bone formation and bone remodeling elucidated by dynamic fluid flow stimulation.

Bone's adaptive response to the dose of fluid flow stimulus was evaluated in this preliminary in vivo experiment using a single frequency of 30 Hz.

Experimental design: The left ulnae of 12 adult, one year old male turkeys were functionally isolated via transverse epiphyseal osteotomies (Fig. 1). A sinusoidal fluid pressure was applied to the ulna in the physiological range at 30 Hz, 10 min/day, for 4 weeks (Table 1).

Table 1. ImP in a disuse model

ImP (mmHg)	N	Physiologic level
15	4	Marrow pressure generated by animal blood pressure
76	4	Close to 700 με peak strain induced marrow pressure
105	4	Pressure induced by bone impact exercise

The adaptive responses of bone were determined through morphometric measurement at the mid-diaphyses; the cortical area of each animal was compared to the contralateral control ulna. The histomorphometry was analyzed by calculating total area adaptation of periosteal and endosteal new surface bone (NB) formation and intracortical porosity using custom designed computer software. The ratio of net change of NB formation was determined by comparing NB and porosity to the area of original bone.

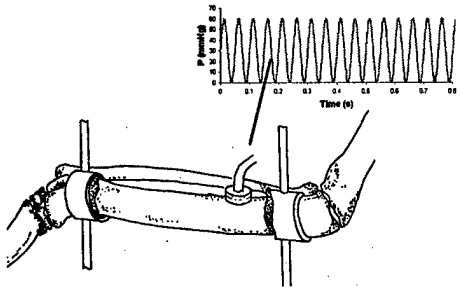

Fig. 1. A diagram of the functionally isolated turkey ulna preparation, such that oscillations in fluid flow can be achieved in the absence of matrix strain. External fixators on the two steel pins at two ends of ulna prevented external mechanical loads. A specially designed device placed into the marrow cavity achieved oscillatory fluid loading. Low magnitude (15 mmHg, 76 mmHg, and 105 mmHg) and high frequency (30 Hz) fluid pressure signal was imposed 10 min daily for 4 weeks in the disuse plus loading group, while the same procedures were prepared for disuse, yet the bone was subject to no exogenous fluid loading.

Results: All animals subjected to fluid flow loading showed a maintenance or gain of total bone mass. While NB formation at the endosteal surface showed no significant difference among applied pressures at 15, 76 and 105 mmHg, the periosteal surfaces did demonstrate dose sensitivity of NB formation. NB increased as a result of an increase in loading pressure, i.e., 2.4±0.3% at 15 mmHg, 5.0±2.0% at 76 mmHg and 8.4±3.7% at 105 mmHg (Fig. 2). Disuse resulted in approximately 3% intracortical porosity. These remodeling experiments have shown nonuniform spatial distribution at the endosteal and periosteal surfaces. Interestingly, increasing ImP did not inhibit intracortical porosity, but rather activated remodeling in the cortex (Fig. 2).

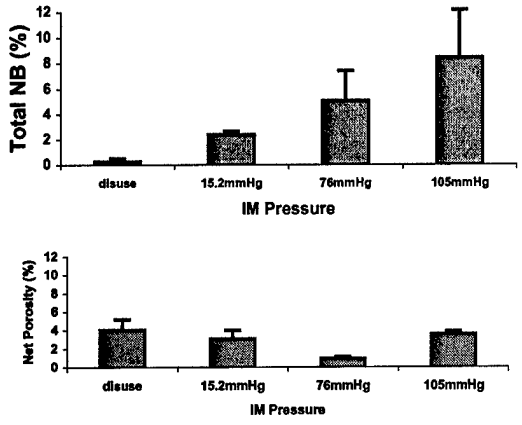

Fig. 2a (top). Histomorphometry of a 100 μm section of the midshaft of ulna analyzed from bones subjected to the disuse control, and ImP stimuli at 15, 76 and 105 mmHg, 10 min per day for 4 weeks. Total new bone calculated by endosteal and periosteal new bone formation, indicated increase as increase of physiological ImP.
Fig. 2b (bottom). 4-week disuse resulted in significant intracortical porosity. Fluid flow stimulation inhibited such bone resorption process only at specific pressure magnitude. i.e., 60~80 mmHg.

Discussion: These data confirm that fluid flow can significantly elucidate adaptive response if applied at proper fluid pressures and cycles or frequency. The results demonstrate that low magnitudes of ImP initiate spatial fluid flow in bone and thus stimulate bone's adaptive response. This suggests that oscillation of ImP can influence the perfusion of bone tissue in many ways. For example, at low physiological levels, ImP induced by circulation alone is on the order of 18 mmHg (2.38 kPa), which will provide basic nutritional supply and fluid pressure gradients to the bone. Resting or inactive of ImP, (i.e., aging, bed rest and microgravity) will influence the fluid perfusion in bone and may substantially stimulate remodeling. At high physiological magnitude, ImP can increase and enhance this perfusion process through increasing fluid pressure. When applied pressure exceeds the physiological intensity or is in the pathologic range, it will trigger an extensive remodeling process and even weaken the quality of bone.

Implication of this work: Loading induced physiologic fluid flow applied to a disuse model can serve as a mediator for increasing both new bone formation and intracortical porosities dependent on the magnitude of the fluid pressure and loading cycles. These experiments may yield new insights into the mechanism, at least at the tissue level, by which dose of bone fluid flow initiates and controls bone morphology and lead to a proper definition of anabolic fluid flow magnitude.

(2) Dynamic ImP induced trabecular bone adaptation

It has been demonstrated that load-induced fluid flow significantly mediates bone mass and morphology in the cortical region. While the fluid stimulus can be controlled quantitatively and potentially applied therapeutically in promoting turnover, the hypothesis of fluid induced trabecular bone remodeling was evaluated in an intact avian ulna model using fluid loading while the animal kept up normal activities on the bone (Fig. 3).

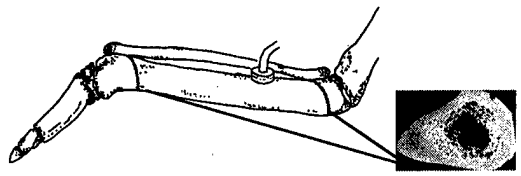

Fig.3. The fluid loader allows a variable frequency and fluid amplitude to be applied inside the bone. The fluid flow was applied to bone in addition to animals' normal activity. Trabecular sections were taken from proximal and distal ends of the ulnae (curved lines), and histomorphometric analysis was used for determining bone's adaptation.

Experimental protocol: The experimental ulna was fluid flow loaded with a pulsatile fluid pressure applied inside the canal at specific frequencies. Three experimental groups were included:
Group A: 30 Hz, 76 mmHg, 10 min/day, 4 weeks

(n=3); Group B: 1 Hz, 76 mmHg, 10 min/day, 4 weeks (n=4); Group C: 30 Hz, 10 mmHg, 10 min/day, 4 weeks (n=4).

On the contralateral ulna, a dummy device was connected into the bone with the same surgical procedure for the sham control. The trabecular regions at both proximal and distal ends were analyzed using histomorphometric measurement (Osteomeasure software, GA) (Fig. 4).

Results: The results reveal an increase of 22.7%±7.2 in trabecular volume [New Bone Volume / Bone Volume (BV/TV)] ($p<0.05$) for group A (30 Hz, 76mmHg) (Fig. 5). BV/TV in Group B (1Hz, 76 mmHg) had only 0.5 % increase between loaded and control bones with no significance. Low magnitude, high frequency, fluid stimulation (Group C, 30 Hz, 10 mmHg) increased BV/TV (15.7%±7.4, $p<0.05$) (Fig. 5).

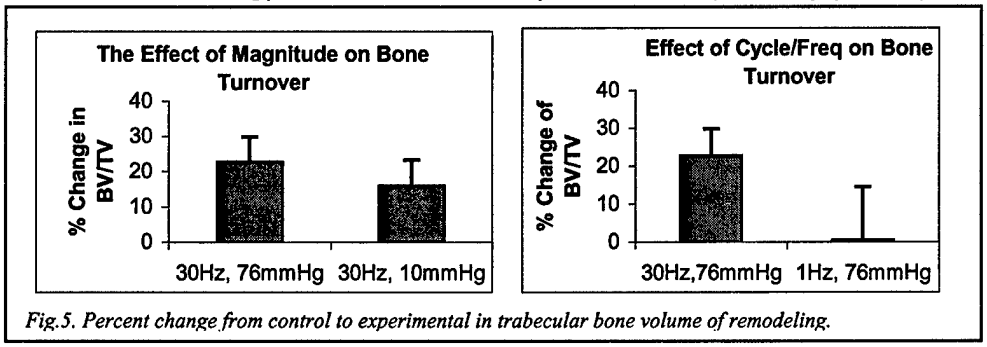

Fig.5. Percent change from control to experimental in trabecular bone volume of remodeling.

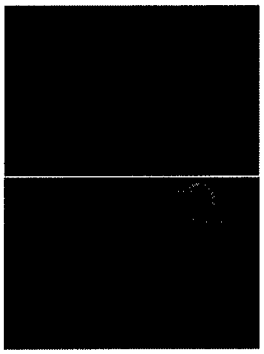

Fig. 4. Applied ImP induced trabecular bone modeling through fluorescent labeling morphometric analysis. Fluid loading generated 23% bone formation (NB) (bottom), while sham control indicate 2.5% new bone formation (above)

Discussion: The data demonstrate that a low magnitude of fluid flow can initiate a sufficient signal for bone's adaptive response in the trabecular region if applied at a proper duration and cycles (via higher frequency). When applying similar physiologic fluid pressure (i.e., 76 mmHg), a high cycle number (18k) of fluid stimuli generate much higher remodeling response (23% bone volume change) than a lower cycle number loading (0.6k) (0.5% BV/TV change). Interestingly, those bones loaded at the high cycle numbers, with smaller fluid magnitude perturbation (i.e., 18K cycles with 10 mmHg) has shown significant bone turnover in the trabecular region (i.e., 16% change of BV/TV). This implies that bone turnover may be more sensitive to the flow cycle number (elevated by frequency) than the pressure amplitude. These data suggest that high cycle of repetitive flow stimuli indeed have strong influence on adaptive process. This may influence fluid perfusion, convection, and surface fluid shear stress.

Implication of this work: This data suggests that fluid flow may, in fact, be the cause of bone remodeling from environmental strain. High cycles of repetitive flow stimuli can have a strong influence on the adaptive process, which may contributes to remodeling induced bone quality reduction.

(3) Repetitive fluid flow is potentially a mediator for pathologic remodeling, and an etiologic factor for osteopenic lesions and stress fractures in an intact model

To evaluate the role of fluid flow as a mediator for pathological remodeling and an etiological factor initiating osteopenia and stress fracture, 12 animals subjected to an experimental protocol.

Experimental protocol: The surgical procedures were performed on intact bones at both left (experimental) and right (sham control) sides of the ulnae as described in (b). By a systematic design of the protocol, a sinusoidal fluid pressure was applied to the left ulna with a magnitude of 70 mmHg, 30 Hz, 10 min/day for two (n=4), three (n=4) and four (n=4) weeks, which resulted in a total cycle numbers of 180k, 270k and 360k, respectively (Table 2). Bone's remodeling response was evaluated by histomorphometric analysis. Weekly radiograph testing was performed to examine the potential fractures in bone.

Table 2. Fluid flow stimulation, 70 mmHg, 30 Hz, 10 min/day

Duration (day)	Total Cycle Number	Observed Cortical lesion & Fracture
28	360k	4/4
21	270k	2/4
14	180k	1/4

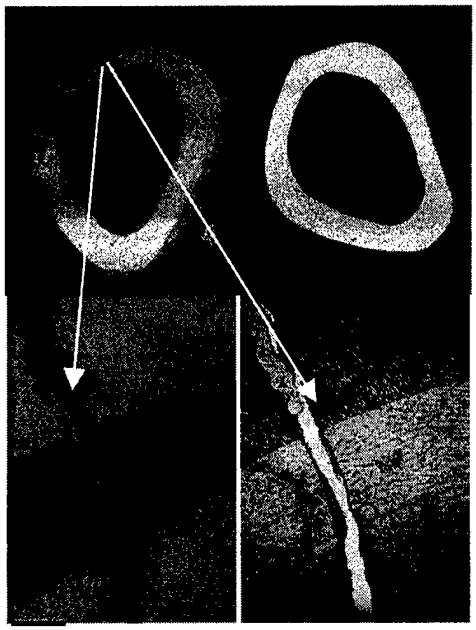

Fig. 6. Tetracycline lables (left-bottom) showed that bone started remodeling (4 wk) at the endosteal surface followed a lesion and ultimately propagate to a fracture. White light image showed the remodeling of cortex (right-bottom) as a part of region of interest from whole cross-section (top-left). Top-right image is the sham control

Results: Four weeks of stimulation showed significant remodeling in the cortex as compared to the contralateral sham control (Fig. 6). 1 of 4 experimental ulnae that underwent two-week of loading observed fracture; 2 of 4 loading animals that underwent three-week duration observed a fracture at experimental ulnae; and all 4 experimental ulnae for four-week of fluid stimulation demonstrated various degrees of fracture (Table 2).

Discussion: These preliminary results suggest that accumulated long duration fluid flow stimulation in an intact animal bone can initiate pathological remodeling. Resultant osteopenia could further weaken bone structure and finally generate cortical lesions and stress fractures. High cycle number was achieved by increasing loading frequency in the animal model, in which the daily cycle number (18k) is similar to the impact exercise created by 1 Hz for 5 hours of continuous marching.

Implication of this work: These results suggest that accumulated long duration fluid flow stimulation in an intact animal bone can initiate pathologic osteopenia, which may further weaken bone structure and ultimately generate fracture.

(4) Reduction of bone blood flow in nutrient vessel by long duration, repetitive, oscillatory fluid loading

In an attempt to evaluate the mechanism that intramedullary pressure stimuli can generate pathological remodeling and lead to cortical lesion, we hypothesized that repetitive fluid flow stimulation can alter marrow cavity blood supply, which will induce partial ischemia through a reduction of nutrient blood supply. This can further trigger the pathologic remodeling in bone. Loading altered blood flow was evaluated in a turkey tibia model.

Experimental protocol: The animal was prepared as one-day terminal experiment on left tibia. Under the general isoflurane anesthesia, the left tibia of an adult, one year old male turkey was exposed at mid-shaft region. Two pins were inserted transcutaneously through the bone to prevent internal and external mechanical loading. The bone was drilled and tapped to provide an insertion of a specially designed fluid loading device allowing ImP oscillation in the marrow cavity. After carefully exposing the nutrient artery, a two-way ultrasonic Doppler probe was mounted to surround the artery close to the inlet entering the tibia. Two triple-rosette strain gauges were mounted on to the bone surface at mid-diaphyses (Fig. 7). Dynamic volume flow and strains were measured and recorded during the entire loading protocol at which oscillatory ImP was applied in marrow cavity continuously for approximately 2.5

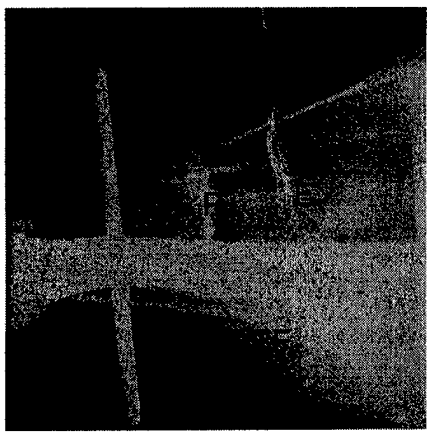

Fig. 7. Oscillatory intramedullary pressure was applied to a tibia model up to 2.5 hours. Fluid pressure (P), bone surface strain (E), and dynamic blood flow (BF) in the nutrient vessel were measured.

hours through various loading frequencies, i.e., 1 Hz, 3 Hz, 10 Hz, 20 Hz, and 30 Hz, and at various pressures, i.e., 15 mmHg, 50 mmHg, 76 mmHg, 100 mmHg, and 125 mmHg. Multiple measurements were performed for fluid pressure, strain, and nutrient blood flow. Data was collected by a pre-amplifier linked to an A/D converter and a computer (Dell PC). Our analog data acquisition card (model AT-MIO-16X, National Instruments, Austin, TX) collected the signals at sampling rate of 1000 Hz with 16-bit resolution for volume flow, ImP and strain.

Results: No strain greater than 1 $\mu\varepsilon$ was observed during the dynamic fluid flow loading. Continuous repetitive fluid pressure decreased the volume flow in the nutrient vessel. The volume flow was reduced by 25 % of original volume after one hour of stimulation (Fig. 8). Even after 15 minutes of rest the full volume of blood did not return to the tibia.

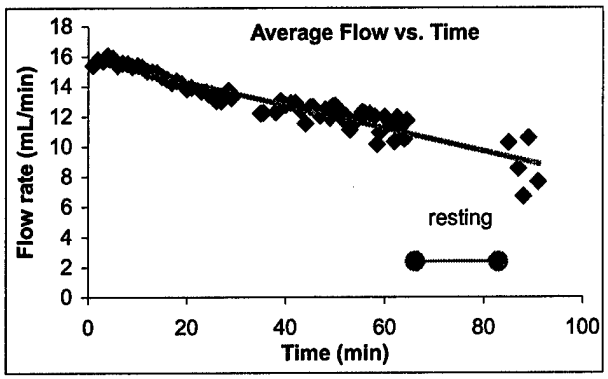

Fig. 8. Volume flow measured from the nutrient artery during ImP loading. Constant repetitive marrow fluid pressure resulted in the reduction of volume flow into the marrow cavity. Short term resting, e.g., 15 min, did not recover the nutrient blood flow to the original rate.

Discussion: These data suggest that the marrow blood supply can be substantially reduced by repetitive, long duration fluid pressure applied in bone. This reduction of flow could elucidate the adaptive response through partial ischemia of the nutrient blood supply of bone and sufficient cycle numbers. The mechanism may include partial closure of marrow vessels in response to the loading, which increases the resistance of the incoming blood flow, even if fluid flow was applied at a physiologic magnitude.

Implication of this work: The results imply that repetitive fluid flow loading may reduce nutritional blood supply and initiates ischemia of bone, which will trigger pathologic remodeling.

(5) Repetitive, long duration, intramedullary fluid pressure oscillatory loading can generate nutrient vessel wall remodeling and potentially reduce blood flow in bone

Arterial vessel wall adaptation to acute or chronic flow changes is proposed to respond to dynamic fluid pressure and fluid shear stress at smooth muscle and the endothelium. Such vessel adaptation in the nutrient artery of bone can be potentially induced by mechanical load generated flow. The objective for this *in vivo* study is to determine the interrelationship between the cyclic hydraulic stimulation in the marrow cavity and the adaptive response of nutrient blood vessels.

Using an avian model, fluid stimuli was applied through sinusoidal fluid loading with the magnitude of 76mmHg at 30Hz, 10 min/day, for 2, 3, and 4 weeks intramedullary in the left ulna, while the right ulna was left unloaded as sham control. The adaptive responses of the nutrient vessels were analyzed through a standard soft tissue histology procedure. The histomorphometry of vessels were examined using a digitized microscope and computerized image processing. The results indicate that the cross-sectional vessel area increases after high cycles of loading, contributed by vessel wall thickening and lumen aeral reduction. The area of the vessel increased as the duration of

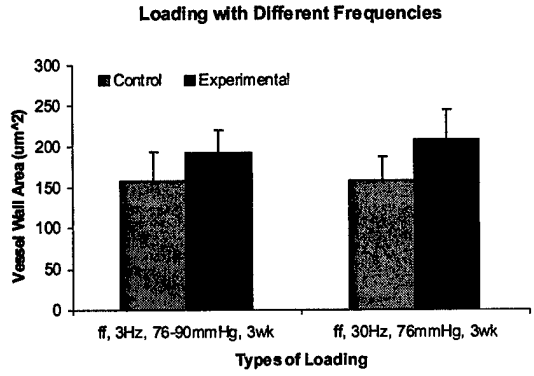

Fig. 9. Histomorphometry of the nutrient vessel cross sections analyzed from ulnae subjected to high repetitive fluid flow (ff) 10 min per day for 3 weeks at 3Hz (1800 cycles/day) and 30Hz (18000 cycle/day). High cycles of fluid flow results in vessel thickening.

loading increased, i.e., vessel area for the 3-weeks of loading is 9% greater than the 2-weeks, and the area for the 4-weeks loading is 36% higher than the 3-weeks and 42% higher than the 2-weeks. This result suggests that repetitive cyclic fluid loading in bone does have an effect on the nutrient vessel adaptation, which may further reduce the blood supply to bone and potentially generate pathological remodeling in bone tissue. This mechanism may partly contribute to certain skeletal diseases, e.g., stress fracture.

Implication of this work: The results imply that repetitive fluid flow loading may generate hypertension in the nutrient vessel and induce vessel wall thickening, and may substantially reduce the nutritional supply of bone.

In summary, these results demonstrated that low magnitudes of ImP could initiate spatial fluid flow in bone and thus stimulate bone adaptive response. At physiological magnitudes, ImP can increase and improve the perfusion process through increasing fluid pressure in the disuse conditions. Within the physiological range, new bone formation is proportional to applied fluid flow stimulation. While the pressure applied exceeds the physiological intensity or falls within the pathologic range, i.e., addition to normal physical loading, it may trigger extensive remodeling and thus even weaken the quality of bone, i.e., increase intracortical porosity and induce lesions. This also suggests that oscillation of ImP may influence the perfusion of bone in many ways. For example, the original marrow pressure, which is induced by circulation, generates marrow pressure on the order of 20 mmHg. It will provide a basic nutritional supply and fluid pressure gradients to the bone. Oscillatory ImP, if applied at the magnitude above the physiologic blood pressure will result in the reduction of the blood supply to bone, which can further result in pathological remodeling and weaken the bone mass.

Summary of the research accomplishments:

(a) Dose dependence of adaptation by fluid flow stimuli in disuse bones

The bone adaptive response to a physiological fluid stimulus, driven by low magnitude, high cycle number (achieved by high frequency) oscillations of ImP were evaluated in a *disuse* avian ulna model, in which fluid pressures were achieved without deforming the cortex. Oscillatory sinusoidal fluid pressure was applied to the ulna with magnitude of 15mmHg (n=4) (magnitude approximates normal marrow blood pressure), 76 mmHg (n=4) (magnitude close to 700 peak microstrain induced ImP) and 105 mmHg (n=4) (pressure close to impact loading) at 30 Hz, 10 min/day, for 4 weeks. The histomorphometry was analyzed by calculating total areal adaptation of periosteal and endosteal new bone (NB) and increase of intracortical porosity (Pore). All animals subjected to fluid flow loading showed surface modeling and intracortical remodeling. While NB formation at the endosteal surface showed no significant difference among applied pressures at 15, 76 and 105 mmHg, it demonstrated dose sensitivity of NB formation at the periosteal surface. This resulted in an increase of NB as a result of an increase of loading pressure, i.e., 2.4±0.3% at 15 mmHg, 5.0±2.0% at 76 mmHg and 8.4±3.7% at 105 mmHg. Disuse and disuse plus loading induced approximately 5%, 3.1%, 1.1% and 3.6% porosity at disuse alone, 15 mmHg, 76 mmHg and 105 mmHg, respectively. Increasing ImP did not inhibit intracortical porosity but rather activated remodeling in the cortex. These data suggested that, under disuse condition, fluid flow can significantly elucidate adaptive response if applied at proper fluid pressure and cycle numbers for a short loading duration. While low magnitudes of ImP can initiate spatial fluid flow in bone and thus stimulate bone adaptive response, high physiological magnitude of ImP can increase this perfusion process through increasing fluid pressure. When fluid pressure applied exceeds the physiological intensity or is in the pathologic range, it will trigger an extensive remodeling process and even weaken the quality of bone.

(b) High cycle number of fluid flow loading generated trabecular bone turnover

The ability of trabecular bone adaptation response to fluid flow stimulation was evaluated in an *intact* animal model, in which animal received fluid flow stimulation in addition to their normal activities. Oscillatory fluid flow was generated in bone in a repetitive manner in turkeys' marrow cavities at 76 mmHg, 10 min per day, at either 1 Hz (600 cycles, n=4) or 30 Hz (18,000 cycles, n=4) for 4 weeks. An additional group was exposed to 10 mmHg, 30 Hz fluid flow stimulation (n=4). The results revealed that the group which received 76 mmHg and 18000 cycles had a turnover increase of 22.7 $\pm$ 7.2% (mean $\pm$ s.d.) in trabecular volume ($p<0.05$), while same magnitude of pressure (76 mmHg) with lower cycle number (600 cycles) resulted only in a 0.5 % increase of trabecular volume. Group of low magnitude (10mmHg) and high cycle (18000 cycles) group had a 15.7 $\pm$ 7.4% trabecular volume increase ($p<0.05$). These results indicate that a high cycle number (elevated by loading frequency) of intramedullary fluid perturbation, if applied near physiologic magnitude, can significantly induce trabecular bone remodeling. Those bones loaded at the same frequency or cycle number, but at a much smaller pressure amplitude, showed similar adaptive response in trabecular bone. This data suggests that fluid flow may, in fact, be the cause of bone remodeling from environmental strain, in which a high cycle of repetitive flow stimuli can have a strong influence on the adaptive process.

(c) Remodeling induced by repetitive fluid flow served as an etiologic factor for osteopenic lesions and stress fracture in an intact model

In an attempt to evaluate the role of fluid flow as a potential etiological mediator initiating osteopenia and stress fracture, 12 animals underwent an experimental protocol. The surgical procedures were performed on intact bones on both left (experimental) and right (sham control) sides of the ulnae. ImP oscillatory stimuli were applied to the left marrow cavity, while the right ulna was served as the sham control. By a systematic design of the protocol, a sinusoidal fluid pressure was applied to the left ulna with a magnitude of 70 mmHg, 30 Hz, 10 min/day for two (n=4), three (n=4) and four (n=4) weeks, which comprise the total cycle numbers of 180k, 270k and 360k, respectively. Bone's remodeling response was evaluated by histomorphometric analysis. Potential fractures were evaluated by weekly radiographs. Four weeks of stimulation showed significant remodeling in the cortex compared to the contralateral sham control. 1 of 4 experimental ulnae that underwent two-week of loading had a fracture; 2 of 4 loading animals that underwent three-week of loading observed fracture in the experimental ulnae; and all 4 experimental ulnae for four-week fluid stimulation demonstrated fractures. These data suggest that cumulated long duration fluid flow stimulation in an intact animal bone can initiate pathologic osteopenia which may contribute to structural weakness and ultimately fracture.

(d) Reduction of bone blood flow in nutrient vessel by long duration, repetitive, oscillatory fluid loading

It is hypothesized in this study that repetitive fluid flow stimulation can alter marrow cavity blood supply, which will induce partial ischemia through a reduction of nutrient blood supply. This will further trigger the pathologic remodeling in bone. Loading altered blood flow was evaluated in a turkey tibia. An animal was prepared as a one-day terminal experiment. Along with the measurements of marrow pressure and bone strain, a two-way ultrasonic Doppler probe was mounted onto the nutrient artery of left tibia. Dynamic volume flow was measured and recorded during the entirety of the loading protocols in which oscillatory ImP was applied in the marrow cavity continuously for approximately 2.5 hours through various loading frequencies, i.e., 1 Hz, 3 Hz, 10 Hz, 20 Hz, & 30 Hz, and at various pressures, i.e., 15 mmHg, 50 mmHg, 76 mmHg, 100 mmHg, and 125 mmHg. The results showed that continuous fluid pressure loading decreased the volume flow into the marrow cavity. The volume flow was reduced by 25 % within one hour of stimulation. 15 minutes of rest was not enough to recover the decrease in volume flow due to the loading. The mechanism may include partial closure of internal marrow blood circulation in response to the loading which increased the resistance of the incoming blood

supply. This can further trigger the pathologic adaptation in bone.

C. Key Research Accomplishments

- Bone's remodeling activity is dependent on the dose of oscillatory fluid flow stimuli

  Increasing ImP under disuse condition, fluid flow can significantly elucidate adaptive response if applied at proper fluid pressure and sufficient loading cycles. While low magnitudes of ImP can initiate spatial fluid flow in bone and thus stimulate bone remodeling response, high physiological magnitude of ImP can increase this perfusion process through increasing fluid pressure. When fluid pressure applied exceeds the physiological intensity or is in the pathologic range, it will trigger an extensive remodeling process and even weaken the quality of bone.

- Trabecular bone turnover is sensitive to the number of cycle of fluid flow loading
  A high cycle number (elevated by loading frequency) of intramedullary fluid perturbation, if applied near physiologic magnitude, can significantly induce trabecular bone remodeling. Those bones loaded at the same cycle number, but at a much smaller pressure amplitude, showed similar adaptive response. This suggests that fluid flow may, in fact, be the cause of bone remodeling, in which a high cycle of repetitive flow stimuli can have a strong influence on the remodeling process.

- Repetitive fluid flow can potentially serve as an etiologic factor for osteopenic lesions and stress fracture in bone

  Four weeks of high cycle of repetitive fluid flow stimulation showed significant remodeling in the cortex, and demonstrated evidence of stress fracture. These suggest that cumulated long duration fluid flow stimulation in an intact animal bone can potentially initiate pathologic osteopenia which may contribute to structural weakness and ultimately fracture.

- Long duration, repetitive oscillatory fluid loading is responsible to the nutrient vessel wall thickening and potentially reduces the blood supply in bone

  Repetitive cyclic ImP has shown an effect on the nutrient vessel remodeling inducing vessel wall thickening dependent on the loading cycle number, duration and pressure magnitude. This may potentially contribute to pathological remodeling in bone.

D. Reportable outcomes

Qin, Y-X., Kaplan, T., Saldanha, A. and Rubin, C.T. (2003): Fluid Pressure Gradient, Arising from Oscillations in Intramedullary Pressure, is Correlated with the Formation of Bone and Inhibition of Intracortical Porosity. J Biomech, (*in press*).

Qin, Y-X., Kaplan, T. (2002): Dose dependence of bone formation and bone remodeling elucidated by dynamic fluid flow stimulation. Ann Am Soc Bone Mine Res, J Bone Min Res, 17:S331.

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Qin, Y-X., Kaplan, T. and Cute, M. (2003): Physiologic oscillatory fluid flow is responsible for bone formation and inhibition of bone resorption dependent on loading magnitude. 49th Ann Mtg Orth Res Soc. J Bone Min Res, 28:110.

E. Conclusions

Bone fluid flow is hypothesized to initiate aberrant remodeling which can ultimately compromise bone quantity and quality. Thus, pathologic response of load-induced fluid flow can potentially damage tissue viability, and initiate bone's remodeling process, ultimately leading to the stress fracture syndrome. Results from the first year's study have shown that within the physiological range, bone formation is proportional to applied fluid flow stimulation. While the pressure exceeds the physiological intensity or falls within the pathologic range, i.e., addition to normal physical loading, it triggers extensive remodeling and thus even weaken the quality of bone, i.e., increase intracortical porosity and induce lesions. Repetitive cyclic fluid flow in bone has shown an effect on the nutrient vessel remodeling inducing vessel wall thickening, which may potentially contribute to pathological remodeling in bone. These results may provide insight of fluid flow in physiological and pathological remodeling and its role in stress fracture.

To understand the etiologic factors of stress fracture is extremely important. As a short-term goal, this study is aimed to acquire an improved understanding of the patho-physiology of stress fractures at the tissue level such as fluid flow alone triggers the osteopenia and lesion. As a long-range goal, if we can identify these osteogenic signals, this may help to design or alter specific training regimes to reduce the risk factors of stress fractures.

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G. Appendices

Qin, Y-X., Kaplan, T., Saldanha, A. and Rubin, C.T. (2003): Fluid Pressure Gradient, Arising from Oscillations in Intramedullary Pressure, is Correlated with the Formation of Bone and Inhibition of Intracortical Porosity. J Biomech, (*in press*).

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Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity

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Abstract

Fluid flow that arises from the functional loading of bone tissue has been proposed to be a critical regulator of skeletal mass and morphology. To test this hypothesis, the bone adaptive response to a physiological fluid stimulus, driven by low magnitude, high frequency oscillations of intramedullary pressure (ImP), were examined, in which fluid pressures were achieved without deforming the bone tissue. The ulnae of adult turkeys were functionally isolated via transverse epiphyseal osteotomies, and the adaptive response to four weeks of disuse ($n=5$) was compared to disuse plus 10 min per day of a physiological sinusoidal fluid pressure signal (60 mmHg, 20 Hz). Disuse alone resulted in significant bone loss ($5.7\pm1.9\%$, $p\leqslant0.05$), achieved by thinning the cortex via endosteal resorption and an increase in intracortical porosity. By also subjecting bone to oscillatory fluid flow, a significant increase in bone mass at the mid-diaphysis ($18.3\pm7.6\%$, $p<0.05$), was achieved by both periosteal and endosteal new bone formation. The spatial distribution of the transcortical fluid pressure gradients (∇P_r), a parameter closely related to fluid velocity and fluid shear stress, was quantified in 12 equal sectors across a section at the mid-diaphyses. A strong correlation was found between the ∇P_r and total new bone formation ($r=0.75$, $p=0.01$); and an inverse correlation ($r=-0.75$, $p=0.01$) observed between ∇P_r and the area of increased intracortical porosity, indicating that fluid flow signals were necessary to maintain bone mass and/or inhibit bone loss against the challenge of disuse. By generating this fluid flow in the absence of matrix strain, these data suggest that anabolic fluid movement plays a regulatory role in the modeling and remodeling process. While ImP increases uniformly in the marrow cavity, the distinct parameters of fluid flow vary substantially due to the geometry and ultrastructure of bone, which ultimately defines the spatial non-uniformity of the adaptive process.

Keywords: Bone fluid flow; Intramedullary pressure; Remodeling; Strain frequency; Osteoporosis; Strain; Stress; Adaptation; Fluid shear stress; Permeability

1. Introduction

Bone's ability to rapidly accommodate changes in its functional environment ensures that sufficient skeletal mass is appropriately placed to withstand the rigors of functional activity, an attribute described as Wolff's Law (Wolff, 1986). The premise of a mechanical influence on bone morphology, now a basic tenet of bone physiology (Lanyon and Baggott, 1976; Carter, 1982; Cowin, 1984; Martin and Burr, 1989; Frost, 1990; Goldstein et al., 1991), indicates that the removal of functional loading is permissive to the loss of bone (Donaldson et al., 1970; Rubin and Lanyon, 1987), while increased activity (e.g., exercise) will result in increased bone mass (Nilsson and Westlin, 1971; Jones et al., 1977; Krolner et al., 1983; Judex and Zernicke, 2000). Considering the strong anabolic potential of mechanical stimuli, and the devastating consequences of removing it, how the bone cell population perceives and responds to subtle changes in their functional environment remains a key issue in understanding the biological and biomechanical processes of bone remodeling. Further, identifying the regulatory components within the mechanical milieu may prove instrumental in devising a biomechanically based intervention for treating osteoporosis, accelerating fracture healing or promoting bony ingrowth.

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In addressing bone's adaptive response to mechanical stimuli, there are a number of different parameters used as the driving function for the optimization process, i.e., strain/stress magnitude, cycle number, number of events, strain tensor and strain energy density. The theories related to strain/stress based bone adaptation range from surface modeling as a function of strain magnitude (Fyhrie and Carter, 1986; Huiskes et al., 1987; Frost, 1990), to time-dependent modeling and remodeling (Beaupre et al., 1990). While there is an overall relationship between intensity of a stimulus and the magnitude of the response, there is very little evidence that the magnitudes of strains or stresses directly correlate to bone's morphological response (Brown et al., 1990; Gross et al., 1997).

It is also important to consider that bone is a highly structured composite material comprised of a collagen-hydroxyapatite matrix and a hierarchical network of lacunae-canaliculi channels. These tunnels permit interstitial flow of fluid through tiny microporosities (Piekarski and Munro, 1977; Weinbaum et al., 1994; Cowin et al., 1995; Cowin, 1999), and thus "by-products" of load, such as the change in fluid velocities or pressures, represent a means by which a physical signal could be translated to the cell (Pollack et al., 1977, 1984; Kelly et al., 1985; Montgomery et al., 1988; Reich et al., 1990; Rubin et al., 1997; Jacobs et al., 1998; Burger and Klein-Nulend, 1999).

To address the potential of this mechanism, load-induced bone fluid flow has been studied both theoretically and experimentally (Pollack et al., 1977, 1984; Gross and Williams, 1982; Montgomery et al., 1988; Reich et al., 1990; Dillaman et al., 1991; Zeng et al., 1994; Weinbaum et al., 1994; Hillsley and Frangos, 1994; Frangos et al., 1996; Jacobs et al., 1998; Tate et al., 1998; Weinbaum, 1998; Burger and Klein-Nulend, 1999; Weinbaum et al., 2001; Mak and Zhang, 2001). Despite the inevitably complex characteristics of fluid flow in porous media (e.g., time and pressure gradient dependent fluid movements), there is early experimental evidence that bone fluid flow driven by loading contributes to the adaptive response, particularly when it is coupled with strain magnitude as well as nutrition supply (Doty and Schofield, 1972; Kelly and Bronk, 1990; Kelly, 1996; Tate et al., 1998). While these experiments demonstrate that cyclic loading can generate significant bone fluid flow as evidenced by streaming potential measurements, there is little evidence to support fluid flow, as opposed to matrix strain, as the driving determinant in bone remodeling, especially under in vivo conditions. This scarcity of data may be associated with the inherent difficulty in separating bone fluid flow induced by mechanical loading from bone matrix strain, as fluid flow certainly is inevitably influenced by bone deformation. However, considering the anabolic potential of low magnitude, high frequency strain (Rubin et al., 2002), and the strong dependence of fluid flow on loading frequency (Qin et al., 1998; Weinbaum, 1998), it becomes essential to determine if these low-level signals derive some of their regulatory potential through fluid flow rather than matrix deformation.

In previous work, we have shown that intracortical fluid flow is induced not only by bone matrix deformation, but also by the intramedullary pressure (ImP) generated during loading (Qin et al., 2002). Further, we have shown that applying anabolic oscillatory ImP alone can induce transcortical fluid flow as measured by streaming potentials (Qin et al., 2000). The principal goal of this study was to test the hypothesis that bone fluid flow, in the absence of matrix strain, can serve as an anabolic stimulus to bone tissue. This goal was achieved by applying low level, high frequency fluctuations in intramedullary pressure in an avian model of disuse osteopenia.

2. Methods

2.1. Animals and experimental preparation

All surgical and experimental procedures were approved by the University's Lab Animal Use Committee. Under general halothane anesthesia, the left ulnae of ten adult, one year old, skeletally mature male turkeys were functionally isolated via transverse epiphyseal osteotomies (Qin et al., 1998). The metaphyseal ends of the ulna were covered with a pair of stainless steel caps and fully sealed with 6 ml of polymethylmethacrylate. Two Steinmann pins, 4 mm in diameter and 92 mm in length, were placed through the predrilled holes in the bone and cap unit. This preparation, including the internal caps, pins and external clamps, can prevent mechanical forces from being applied during daily activities, effectively serving to isolate the bone from any mechanical strain. A 4-mm diameter hole was drilled through the cortex at the dorsal side, approximately 1.2 cm from the proximal cap. The hole was tapped and a specially designed fluid loading device, with an internal fluid chamber approximately 0.6 cm^3 in volume, was firmly connected to the bone with an O-ring seal (Fig. 1). A diaphragm was included in the center of the chamber dividing the internal marrow fluid from the external oscillatory loading flow. A surgical plastic tube (2-mm inner diameter), which was connected to the device and passed through the skin, served to couple the device fluid chamber and the external fluid oscillatory loading unit. An injection plug (Terumo Medical Co., Elkton, MD) was connected to the external end of the tube to facilitate fluid flow loading. With the diaphragm and the injection plug, the bone marrow and oscillatory flow media were fully isolated

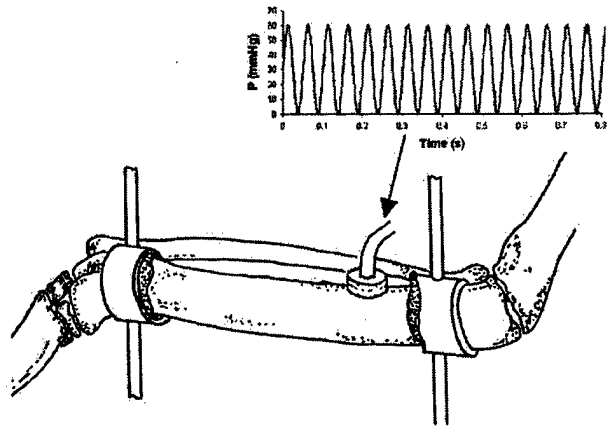

Fig. 1. A diagram of the functionally isolated turkey ulna preparation, such that oscillations in fluid flow can be achieved in the absence of matrix strain. External fixators on the two steel pins at two ends of ulna prevented external mechanical loads. A specially designed device connected into marrow cavity achieved oscillatory fluid loading. A low magnitude and high frequency (60 mmHg, 20 Hz) fluid pressure signal was imposed 10 min daily for 4 weeks in the disuse plus loading group, while the same procedures were prepared for disuse, yet the bone was subject to no exogenous fluid loading.

from the external environment to prevent any infection. To monitor the bone remodeling response, all animals were labeled weekly using tetracycline solution (15 mg-Kg^{-1}) through IV. The contralateral ulna served as control.

In addition to the 10 experimental animals, fluid flow was validated via two animals used to calibrate the loading device and its induced pressure magnitude with varied frequencies. The same surgical procedure was used in these animals. An additional tube was connected to the distal end of the ulna. Through this tube, a 50-psi pressure transducer (Entran EPX-10IW) was connected into the medullary canal, thus permitting measurement of the intramedullary pressure during animal rest and applied external ImP loading. The marrow pressures were recorded within the physiological pressure magnitude, 10 ~ 180 mmHg, and at a variety of frequencies, 1 ~ 40 Hz. The marrow pressure elevated by imposing ImP was then used to calibrate the loading system.

2.2. Dynamic fluid flow loading

The animals remained under close supervision until recovery from anesthesia and extensively monitored for an additional 2 h to make certain that they were able to stand normally and resume normal activities. Controlled fluid pressure oscillations started on day two after the surgical preparation. A sinusoidal fluid pressure was applied to the marrow cavity of the ulna at a peak magnitude of 60 mmHg at 20 Hz for 4 weeks ($N = 5$). The remaining animals ($N = 5$) were subject to an identical surgical procedure, except that there was no fluid pressure loading, and thus served to represent disuse.

2.3. Quantification of bone remodeling

Following a 10-min period of loading each day for 4 weeks, animals were euthanized via a bolus IV injection of saturated barbiturate. The ulnae of experimental and contralateral control were dissected free of soft tissue, and fixed for 48 h in 70% ethyl alcohol. After dehydration, the pair of ulnae for each animal was carefully positioned in a plastic box and embedded using polymethylmethacrylate.

2.3.1. Areal properties

Approximately 100 μm thick sections were cut from the midshaft of the ulnae using a precision diamond wire saw (Well Walter, Model 3241). Each section was microradiographed, scanned at a resolution of 600 dpi with a high-resolution film scanner (Minolta Dimage Scan Multi, Model F-3000, Japan), and converted to a binary image. The final image resolution was approximately 10 μm/pixel. The cortical area of each fluid loaded ulna was compared to the contralateral control ulna depending on the geometric similarity of the pair of the ulnae (Adams et al., 1995). Endosteal and periosteal new bone formation, as well as intracortical porosity, were traced using custom-written programs (PV-WAVE, Visual Numerics, Boulder, CO). Changes in bone mass, sites of new bone formation and porosity were determined by comparing the adapted area to the bone morphology of the contralateral control ulnae. Since the periosteal surface circumference remained unchanged in disuse bones, morphometric changes were determined by calculating areal differences between contralateral control and disuse alone in each animal. The initial starting point and the orientation of the sectors were based on the orientation of the ventral cortex in the ulna. This orientation is consistent with the turkey ulna anatomy.

In addition to total new bone formation, resorption and porosity changes were approximated using sector analysis in which the bone cross-section was divided into twelve equal angle (30°) pie sectors through the centroid of the bone section (Fig. 2) and compared to the animal's contralateral control. The number of sectors was selected by referring to previous studies, e.g., sectors ranged from 6 to 24 (Gross et al., 1997; Judex et al., 1997; Qin et al., 1998). The transcortical fluid pressure gradient was then calculated for each sector as the difference between fluid pressures at the endosteal and periosteal surfaces, where fluid pressure at the periosteal surface was considered zero. More specifically, the pressure gradient was estimated by dividing the averaged linear distance between the periosteal and endosteal surfaces using a total of 30 pairs of points in each

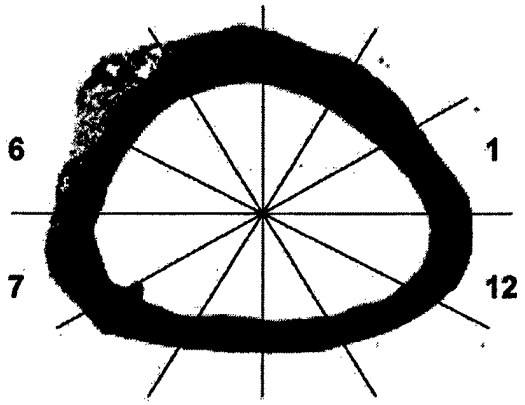

Fig. 2. A mid-diaphyseal cross-section is shown divided into 12 equi-angle sectors. Areal analyses were used to calculate surface bone mass changes and intracortical porosity in each sector. Bone mass changes were evaluated by comparing bone's adaptive responses between an experimental ulna and its contralateral control, as well as within and across experimental groups. An averaged transcortical pressure gradient was calculated in each sector by the pressure difference between endosteal and periosteal surfaces.

sector, where the relation can be expressed by the equation:

$$\nabla P_r = [\mathrm{ImP}]_{\mathrm{peak}} * [\text{cross cortical distance}]^{-1}$$

2.3.2. Histomorphometry

Quantification of the cortical modeling/remodeling response to these two distinct stimuli was determined using the distribution of the fluorochrome labels. Histomorphometric evaluation of undecalcified diaphyseal, metaphyseal and epiphyseal sections was performed on a Nikon Labophot system including epi-fluoresence microscopy, and reflected light microscopy. The fluorescent photomicrographs were taken through the microscope (× 10) and the photographs digitized at 600 dpi using a high resolution SONY digital CCD camera (Model DXC-950P, Japan). The final image resolution was approximately 1.1 μm/pixel. The image processing was performed using custom-written programs in PV-WAVE. Initial conditions were considered to be the labeling status of the contralateral control bone (Adams et al., 1995). The total area of new labeling and its transcortical distribution were then determined using imaging analysis in PV-WAVE. The magnitude and site specificity of disuse or disuse plus the oscillating fluid flow was determined by quantifying new bone formation at endosteal and periosteal surfaces, as well as porosities within the intracortical region.

2.3.3. Statistical analysis

Differences between disuse and disuse plus fluid flow were analyzed using paired student t-test. Significance was considered at $p \leqslant 0.05$. Linear regression was used to identify the relationship between the distributions of fluid parameters and the spatial modeling/remodeling parameters in bone in the fluid flow loading group using t-test of the linear regression (Watson, 1992). Thus, the significance of fluid flow on bone was tested in three ways: first, by determining the effective differences between experimental and contralateral control ulnae in both disuse and disuse plus fluid flow groups; second, by testing the significance between disuse and disuse plus fluid flow groups; and third, by correlation between fluid pressure gradient and site-specific response of adaptation in the fluid loading group. A paired two-sample student's t-test was performed to determine whether a sample's means were distinct from other criteria.

The contralateral controls in disuse and disuse plus fluid-loading groups served as an intra-animal control. Bone loss caused by disuse was evaluated by comparing experimental and contralateral control ulnae. The fluid-loading group had undergone the same surgical procedure and under the same experimental period as the disuse group. While intra-animal comparison is more accurate and more effective than cross animal comparison, the results of net bone adaptation, morphological loss or gain, were obtained from intra-animal paired data. The final results were explored between groups; between intra-animal experimental and control.

3. Results

3.1. Changes in areal properties

Morphometric changes in the group subject to disuse alone for 4 weeks indicated a significant loss of bone in the mid-diaphyseal cross sectional area; primarily due to an increase in the percent of the total bone envelope which was porotic ($5.7 \pm 1.9\%$, mean ± s.d.; total porosity vs. total bone area) as compared to that measured in the contralateral control ($1.6 \pm 0.7\%$; $p = 0.05$) (Fig. 3a,b). There was no evidence of bone resorption at the periosteal surface in any animal. Total area of bone mass (including the area of porosity) in the animals subject to 4-week disuse remained similar between disuse and contralateral control, yielding total cross-sectional areas of $53.8 \pm 3.5\,\mathrm{mm}^2$ (mean ± s.d.) and $52.7 \pm 2.5\,\mathrm{mm}^2$, respectively. Morphometric analysis showed that 10-min per day of the oscillating fluid flow resulted in a significant increase in bone mass at the mid-diaphysis ($18.3 \pm 7.6\%$; total new bone/total bone area) ($p < 0.05$), primarily due to periosteal ($16.1 \pm 6.5\%$, $p < 0.05$), as opposed to endosteal ($2.2 \pm 1.6\%$, $p = 0.13$) new bone formation (Fig. 3c).

The sites of modeling and remodeling response in bone were consistent using microradiograph and fluor-

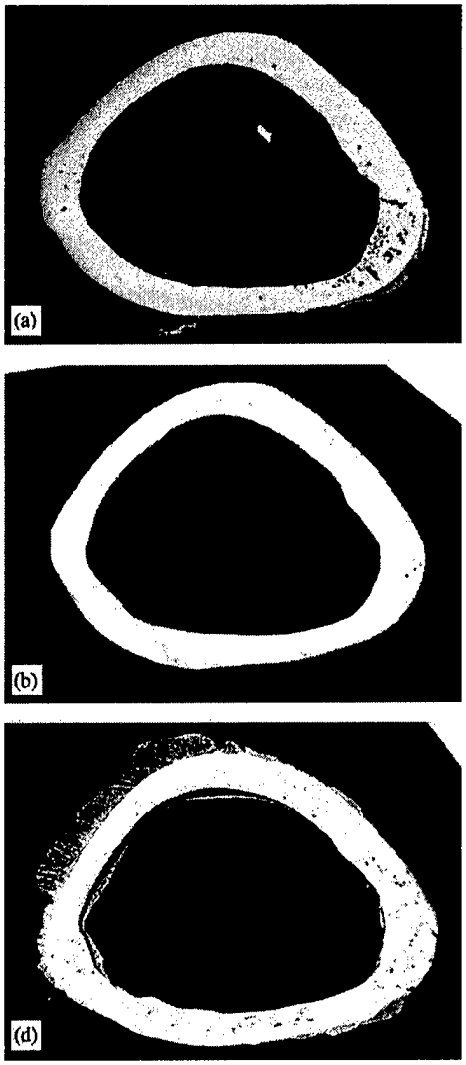

Fig. 3. Microradiographs of (a) animal subject to 4-week disuse resulted in significant bone loss by increase of intracortical porosity. (b) contralateral control of disuse ulna (a). (c) 4-week fluid flow loading resulted in significant new bone formation in periosteal and endosteal surfaces, yielding total of 18% new bone formation as compared to control.

escent labeling analyses, in which new bone formation and intracortical remodeling were identical at the locations of the adaptive response.

3.2. Sector specific stimulation of bone adaptation

In animals subject to the fluid pressure oscillations, despite a uniform marrow pressure at the endosteal surface, oscillatory ImP generated non-uniform spatial distributions of transcortical fluid pressure gradients through the cortex, rising 37% from $4.9 \pm 0.2\,\text{kPa-mm}^{-1}$ in sectors 1 & 7, to $6.7 \pm 0.4\,\text{kPa-mm}^{-1}$ in sectors 5 & 6 (Fig. 4).

Although the ImP stimulus was ultimately non-uniform about the cortex, the non-uniform patterns were consistent between animals. At the periosteal surface, maximum new bone formation was observed in sectors 4, 5 and 6, yielding new bone formation of $1.7 \pm 0.6\,\text{mm}^2$, $3.1 \pm 1.0\,\text{mm}^2$ (25% & 41% gains, respectively), and $2.8 \pm 1.2\,\text{mm}^2$ (39% gain) ($p < 0.02$), respectively (Fig. 5). The area of endosteal new bone gain in each sector showed an average gain of $0.13 \pm 0.08\,\text{mm}^2$ ($p = 0.13$), ranging from a maximum gain of $0.5 \pm 0.3\,\text{mm}^2$ (7.8% gain in sector) in sector 4, to zero change in sectors 1 & 8. There was no significant difference of endosteal new bone formation among sectors. Oscillatory ImP stimuli resulted in increase of porosity area in each sector from maximum increase of $0.37 \pm 0.12\,\text{mm}^2$ (6.3%) in sector 12, to a minimum increase of $0.17 \pm 0.05\,\text{mm}^2$ in sector 5 (Fig. 5).

3.3. Correlation between fluid pressure gradient and bone formation

A strong correlation was observed between the transcortical fluid pressure gradient, ∇P_r, induced by oscillatory ImP and periosteal new bone formation ($r = 0.77$, $p = 0.01$), as well as total new bone formation ($12.1\,\text{mm}^2$ gain with 18.3% increase, $p \leqslant 0.01$) (Figs. 4 and 6). Endosteal new bone formation was weakly correlated with the ∇P_r ($r = 0.53$, $p = 0.08$). Interestingly, a negative correlation ($r = -0.75$, $p = 0.01$) was found between increased area of intracortical porosity and ∇P_r (Fig. 6).

4. Discussion

To determine the regulatory influence of bone fluid flow on the adaptive response of bone, it is necessary to gather both in vitro and in vivo data from a variety of fluid flow conditions. In the past few years, many studies report proliferative responses of osteoblast-like cells, and inhibiting effects on osteoclast-like cells, under pulsing or oscillatory fluid flow loading in culture (Frangos et al., 1996; Rubin et al., 1997; Jacobs et al., 1998; Burger and Klein-Nulend, 1999). For example, oscillatory flow resulted in greater cellular responses than steady flow (Jacobs et al., 1998). These studies unveiled a cellular response to fluid flow loading in an in vitro environment. However, it is possible that the fluid magnitudes examined in these studies were not entirely consistent with those levels which are a fluid representative of physiological levels, e.g., at relatively high pressure magnitudes and at great fluid shear stresses. Further, many studies use immature cells or cells from very young animals, which may not reflect bone cell behavior which could be expected in adults under the conditions of normal mature cell. Finally, it is

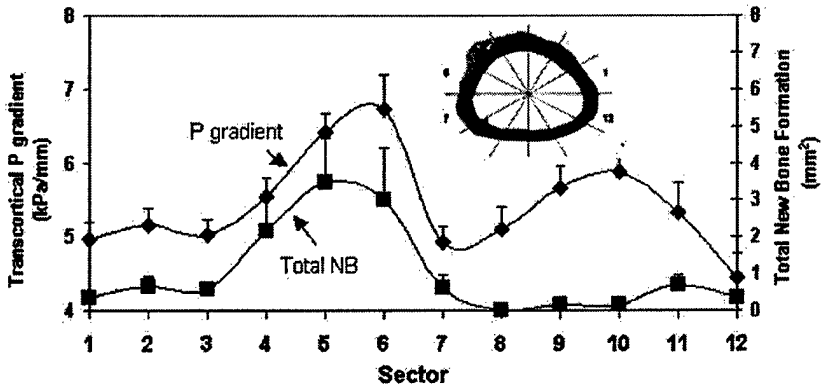

Fig. 4. Mean value (±s.e.) of transcortical fluid pressure gradient distributions for each of 12 sectors in the animals subject to fluid flow loading. Maximum pressure gradient was observed in sector 6, which corresponded to the site of maximal new bone formation. Minimum pressure gradients were located corresponding to least new bone formation sectors, i.e., sector 12 & 7.

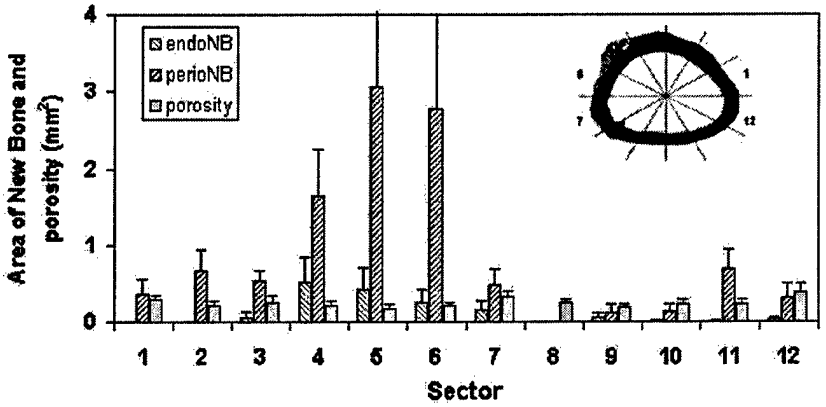

Fig. 5. Spatial distributions, mean (±s.e.), of new bone formation at periosteal and endosteal surfaces as well as an increase in intracortical porosity in each sector. ImP resulted in significantly new bone formation, achieved primarily by periosteal new bone formation ($p<0.02$). Fluid flow loading also minimized intracortical porosity.

important to consider that in vitro experiments, while proving insight into the mechanism of a cellular response, do not ultimately indicate whether new bone will be formed, or that bone loss can be inhibited. This suggests that knowledge of how bone cells accommodate a systematic, physiologic and morphologically "appropriate" fluid flow environment is important to address the relevance of these signals in controlling bone's adaptive response. In an attempt to examine the anabolic potential of fluid flow loading in vivo, this study applied oscillatory intramedullary fluid pressure at low magnitude and high frequency, where it was found to stimulate bone formation and reduce bone porosities caused by disuse. This flow stimulus is considered to be physiological in the in vivo flow environment. Thus, if fluid flow is "sufficient," it is capable of stimulating new bone formation and inhibition of bone resorption with only a daily 10 min period of loading. This implies that physiological fluid flow is indeed a mediator critically involved in bone modeling and remodeling, and that its influence can be realized in the absence of matrix strain per se.

Given the porous nature of bone, the fluid filled spaces invariably generate a flow upon mechanical loading. In general, load-induced flow and its associated matrix strain are usually coupled. Therefore, segregating the regulatory potential of matrix strain from the anabolic potential of fluid flow becomes inherently difficult. Matrix strain, as a general parameter of bone receiving mechanical loading, is commonly used in describing bone tissue deformation. If bone fluid flow is indeed a key mediator for bone modeling and remodeling, then it is important to test the accommodation of tissues and cells to a customary flow loading environment. This study tried to separate matrix strain and convective fluid flow by dynamically pressurizing the marrow cavity which drives interstitial fluid to flow. The fluid magnitude for such a flow remained in the physiological range generated in the marrow cavity by an animal's normal activities (Fritton et al., 2000).

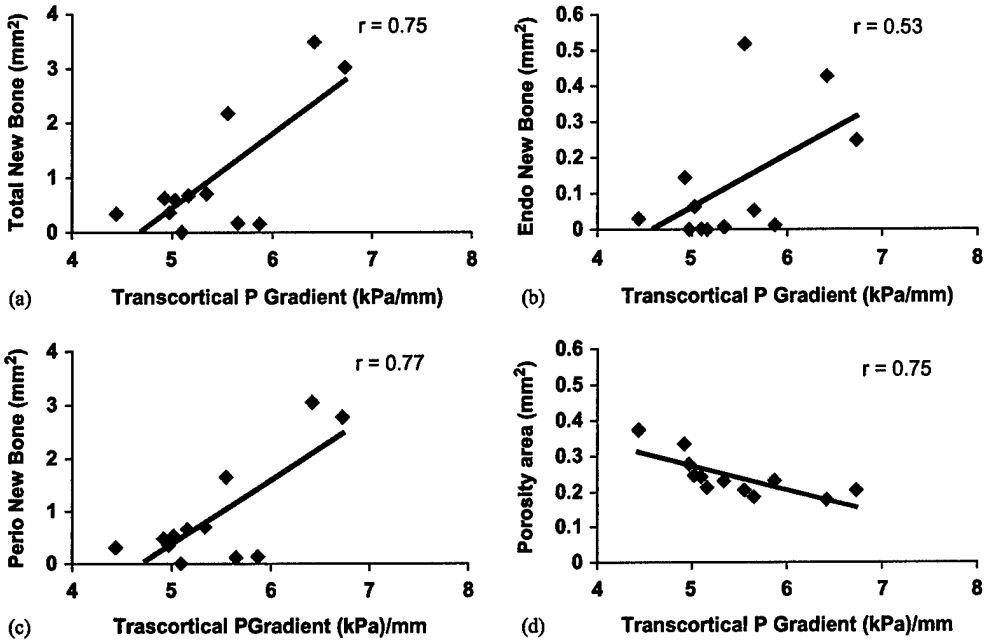

Fig. 6. The strong correlation between calculated pressure gradients and total new bone ($r=0.75$, $p<0.02$) (a). While a weak correlation was observed between endosteal new bone formation and pressure gradients ($r=0.53$) (b), a strong correlation was observed between transcortical fluid pressure gradients and periosteal areal adaptation ($r=0.77$, $p<0.02$) (c). A negative correlation was identified between increase in intracortical porosity and increase of transcortical pressure gradients ($r=0.75$, $p<0.02$) (d).

When dynamic hydraulic pressure is pressurized in the marrow cavity and interstitial pore space, however, there was concern that the imposed ImP would create deformation in the matrix. Assuming a solid material modulus of 10 GPa and isotropic elastic mechanical behavior of cortical bone, it is estimated that a maximum fluid pressure on the order of 8 kPa will result in approximately 0.8 με in the matrix. Further, assuming a strain–stress relation in a poroelastic model and a bulk modulus of 5 GPa for the two-phase material, then the calculated matrix strain falls to less than 0.1 microstrain (Neidlinger-Wilke et al., 1994; Cowin, 1999). It is difficult to envision a physical mechanism by which ImP loading resulted in new bone formation could be generated by such small matrix strain, particularly in light of the strong in vitro evidence that fluid flow can perturb to the biological response of bone cells. This experiment suggests that fluid flow can, in and of itself, influence parameters of bone formation and resorption.

The sites of greatest osteogenic response correlated with the greatest gradient of transcortical fluid pressures. The strong correlation between new bone formation and fluid flow suggests that fluid components, i.e., pressure gradients, a close source driving fluid velocity and fluid shear stress, may directly influence the response of bone cells to mechanical stimulation. In addition, the correlation between minimal intracortical porosity and elevated fluid pressure gradients implies that a basal level of convectional bone fluid flow is critical in preserving cortical mass against disuse, such as conditions of bed rest and microgravity. At the very least, it is clear that extremely low-level perturbations of fluid flow, as induced by high frequency oscillations, are providing necessary signals to inhibit intracortical porosity and stimulate new bone formation. Given the anabolic potential of these high frequency signals (Rubin et al., 2002), and the rapid rise in fluid velocities that occur because of high frequencies even in conditions of very low strain (Weinbaum, 1998; Weinbaum et al., 2001), it is certainly possible that signaling the cells responsible for orchestrating bone adaptation is achieved not by subtle changes in matrix strain, but by changes in fluid flow.

The implication of the strong correlation between fluid flow components, i.e., pressure gradients, driven by ImP, is interesting because of its potential to impose fluid shear stress in the cellular environment. A number of theoretical models have been proposed to describe a potential mechanism of fluid pressure and fluid shear stress in bone (Dillaman et al., 1991; Weinbaum et al., 1994; Cowin et al., 1995; Mak et al., 2000), which have been supported by mounting in vitro experimental work (Frangos et al., 1996; Jacobs et al., 1998; Burger and Klein-Nulend, 1999). The effects of an increase in fluid flow induced by oscillatory ImP can potentially influence bone cell activities through several coupling mechanisms. First, raising the ImP can result in a

corresponding increase of outward fluid flow through various fluid pathways, which include the vascular system and the extensive lacunar–canalicular spaces in which the bone cell population resides. Increased fluid velocities can produce fluid shear stresses on the endothelial lining cells of vessels (Girard and Nerem, 1995) and on the bone cells in the lacunar spaces (Weinbaum et al., 1994), where the oscillatory ImP can alter the fluid shear stresses on the cell population and trigger a cellular response. Second, a nutrient pathway for metabolism and the proper disposal of waste products generated from catabolic activities occur through fluid channels. In the soft tissue, molecular diffusion is considered the major pathway for transportation of metabolites (Otter et al., 1999). Because of the relatively dense structure of cortical bone, however, the diffusive mechanism may, in fact, be insufficient to play an adequate role in transporting metabolic constituents between osteocytes and the surrounding vascular canals. An imposed dynamic ImP will enhance this fluid transportation from the blood supply to osteocytes through this convective perfusion mechanism (Piekarski and Munro, 1977; Tate et al., 1998; Wang et al., 2000), where the greatest exchange occurs at sites of greatest pressure gradients.

The fluid magnitude for ImP stimulation was imposed at physiological levels. Via Haversian canals, fluid flow can be modulated by blood flow during ImP stimulation. Blood supply to the long bone is achieved through both marrow and periosteal nutrient vessels, with the main vessel dividing before entering the marrow cavity. The blood supply via the marrow is primarily, but not entirely, centrifugal (Brookes, 1967; Singh and Brookes, 1971; Slater et al., 1991; Churchill et al., 1992; Kiaer, 1994; Bridgeman and Brookes, 1996). The marrow pressure caused solely by the circulation is on the order of 10 to 50 mmHg, depending on the species (Kumar et al., 1979; Bryant, 1983; Otter et al., 1999), and is approximately 18 mmHg in the turkey ulna. While mechanical fading can increase marrow pressure on the order of 150 mmHg with 800 ~ 1000 microstrain axial loading, ImP oscillations which can attain pressures of 60 mmHg, would incorporate with the circulatory blood pressure and impact on bone fluid flow. Enhanced fluid convection dependent on either transcortical flows through particular fluid perfusion pathways or altering basal blood flow is supported by the non-uniformity of the surface bone formation.

While physiologic fluid flow showed the potential to initiate the modeling and remodeling process, dynamic components of this fluid may also play an important role in the regulation of adaptation. It is recognized that bone tissues respond very differently to static vs. dynamic load environments, and results in an adapted structure which demonstrates similar peak strain magnitudes during vigorous activity (Lanyon and Rubin, 1984). These regulatory "temporal" components may include strain rate, strain frequency, and strain gradients (O'Connor et al., 1982; Rubin and McLeod, 1994; Turner et al., 1995; Gross et al., 1997; Qin et al., 1998). These temporal components result not only in local matrix deformation, but also in fluid flow, streaming potentials, and other physical phenomena, which also influence cell responses. For example, in the case of the turkey ulna, 10 min of loading per day at 1 Hz requires a peak induced longitudinal normal strain greater than 700 $\mu\varepsilon$ to maintain bone mass, while a relatively high frequency (30 Hz) loading regimen reduces this threshold to 70 $\mu\varepsilon$ (Qin et al., 1998). The stimulatory effects of fluid flow, driven at physiological magnitude and high frequency but with minimal matrix strain, may depend on the cellular response due to (1) intermittent rather than static flow constant velocity, (2) direct fluid shear stress perturbation, (3) the cumulative effect of small local fluid movements resulting in cells accommodating to large flow cycles, and (4) even an "amplified" effect on the bone cell which could result in pressurization and/or fluid shear stress on the cell (Weinbaum et al., 2001). Again, these data, while not intended to diminish the role of bone strain, imply that anabolic fluid flows, applied in a dynamic manner, can have a tremendous influence on bone mass and morphology even under conditions of extremely low matrix deformations.

That fluid flow results in periosteal expansion in response to intramedullary pressure and transcortical pressure gradients help identify a physical mechanism for the response. Since the periosteum is often referred to as an impermeable layer for fluid perfusion, it is understandable that periosteal modeling requires fluid exchange and/or flow to initiate such an adaptive process. Fluid flow resulted in periosteal bone formation in this study, and thus implies that oscillations of ImP influence bone fluid perfusion and convection in many ways. While the endosteal surface provides an open circulation between marrow pressure and intracortical flow, the interstitial fluid flow in bone must flow out of the mineral to the periosteal surface through a variety of fluid pathways (Morris et al., 1982; Tate et al., 1998; Wang et al., 2000). Since the loading pattern used in these experiments was oscillatory, it may not be necessary that fluid physically flowed out of the periosteal surface but, instead, the oscillation itself may serve as a stimulatory signal. Under oscillatory fluid stimulation, however, a local fluid pressure gradient may be built up with the semi-permeable periosteal boundary condition which will create a flow at the periosteal surface. The spatial distributions of such fluid flow patterns ultimately is dependent on the fluid pressure gradients, defined somewhat by the geometry, ultrastructure and fluid pathways of the bone.

Fluid pressure gradients were calculated based on the assumption of zero pressure magnitude at the periosteal

surface boundary. Using a poroelastic two-phase FEA model, we have previously calculated that transcortical fluid pressure gradients at the periosteal surface were relatively similar when considering either impermeable or semi-permeable surface boundary conditions (Qin et al., 2000). In this particular case, the calculated transcortical pressure gradients may remain proportional regardless of the periosteal permeable conditions, e.g., impermeable vs. semi-permeable. This suggests that the correlation between the morphometric bone formation and the calculated transcortical fluid pressure gradients may be consistent for different permeabilities of the periosteum. However, to better understand interstitial fluid movement, identifying the hydrostatic permeability of cortical bone and the surface boundary conditions are indeed critical. It was found that bone permeability was dependent on many factors, i.e., age and spatial location. Using a canine tibia model, the permeability of puppy tibiae is six times higher than that of adult tibiae (Li et al., 1987). The high permeability of their cortical bone may explain the increase in periosteal new bone formation seen in puppies when a venous tourniquet is applied. While the endosteum is permeable, they have found that the periosteum is, in essence, impermeable unless the periosteal superficial layer is removed in the adult canine tibial cortex. Many tracer studies have indicated that fluid perfusion can penetrate both endosteum and periosteum. Penetration of bone fluid can be greatly enhanced by convection through mechanical loading. It was observed that, in loaded bone, the concentration of tracer dispersed through the mid-diaphysis and surface of the cortex was significantly higher than that which was measured in the unloaded bone (Tate et al., 1998). Nevertheless, to identify the periosteal permeability in this model will help to understand the fluid flow pattern in bone through the convection mechanism. This may be important for future work.

There were several other limitations in the study. Like many in vivo studies, this protocol required an invasive surgical preparation, which potentially altered the bone metabolism and introduced complications during fluid loading. To minimize the variability that inevitably occurs in a biological system, the influence of the surgical procedure might, to a certain degree, be determined by the sham group and the contralateral control. In addition, the fluid loading hole was located on the dorsal side of the ulna. The hole was physically 3 cm away from the midshaft where the cross sectional morphometric analysis was performed. This distance exceeds the threshold of a surgical procedure inducing bone modeling/remodeling activity. In addition, there were distinct limitations in relying on the analytical analysis, in which it is necessary to simplify what is undoubtedly a complex biological system. There are several fluid filled spaces in the cortical structure, e.g., Haversian channel, lacuna-canaliculi, and micropore space. The calculation of fluid pressure gradients was based on the assumption of uniform distributions of these porosities in bone. Although accurately determining the true distributions of fluid channels and porosity of cortical bone is immensely difficult, the potential inaccuracy can be modified with further determined spatial distribution of pore size and permeabilities.

In conclusion, in an effort to determine how bone tissue senses fluid flow related stimuli, and their importance to the adaptive response in bone, we have developed an animal model which can induce oscillatory fluid flow in the absence of bone matrix deformation. The results indicate that small perturbations of basal fluid flow can influence both bone formation and resorption. New bone formation on the periosteal surface was strongly correlated to fluid pressure gradients, suggesting the adaptive response to be influenced by both fluid velocity and shear force. These data also suggest that maintaining anabolic cortical fluid flow is essential to maintain intracortical bone mass against the effects of disuse. The results imply that the fluid flow induced by physiological values is essential and important in retaining bone quality and quantity, and that small fluctuations in fluid flow, achieved via pressure differentials, has potential for therapeutic applications against skeletal disorders even in the absence of mechanical strain.

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Dose dependence of bone formation and bone remodeling elucidated by dynamic fluid flow stimulation

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Introduction: Fluid flow that arises from the functional loading of bone tissue has been proposed to be a critical regulator of skeletal mass and morphology. To test this hypothesis, the bone adaptive response to a physiological fluid stimulus, driven by low magnitude, high frequency oscillations of intramedullary pressure (ImP) were examined, in which fluid pressures were achieved without deforming the bone tissue.
Methods: The left ulnae of 12 adults, one-year-old male turkeys were functionally isolated via transverse epiphyseal osteotomies. An oscillatory sinusoidal fluid pressure was applied to the ulna with the magnitude of 15mmHg (n=4) (magnitude approximated to the normal marrow blood pressure), 76 mmHg (n=4) (close to 700 peak microstrain induced ImP) and 105 mmHg (n=4) (pressure close to impact loading) at 30 Hz, 10 min/day, for 4 weeks. The adaptive responses of bone were determined through morphometric measurement at the mid-diaphyses. The histomorphometry was analyzed by calculating total area adaptation of periosteal and endosteal new surface bone (NB) formation and intracortical porosity.
Results: All animals subjected to fluid flow loading showed a maintenance or gain of total bone mass. While NB formation at the endosteal surface showed no significant difference among applied pressures at 15, 76 and 105 mmHg, it demonstrated dose sensitivity of NB formation at the periosteal surface. This resulted in an increase of NB as a result of an increase of loading pressure, i.e., 2.4±0.3% at 15 mmHg, 5.0±2.0% at 76 mmHg and 8.4±3.7% at 105 mmHg. Disuse induced approximately 3% intracortical porosity. These remodeling experiments have shown nonuniform spatial distribution at endosteal and periosteal surfaces. Interestingly, increasing ImP did not inhibit intracortical porosity rather it activated remodeling in the cortex.
Discussion: These data confirmed that fluid flow could significantly elucidate adaptive response if applied at proper fluid pressure and frequency in a short loading duration. The results demonstrated that low magnitudes of ImP can initiate spatial fluid flow in bone and thus stimulate bone adaptive response. At high physiological magnitude, ImP can increase and improve this perfusion process through increasing fluid pressure. Within physiological range, new bone formation is proportional to applied fluid flow stimulation. While pressure applied exceeds the physiological intensity or in the pathologic range, it will trigger extensive remodeling process and even weaken the quality of bone.

INTERRELATIONSHIP BETWEEN RATE AND MAGNITUDE OF FLUID FLOW AND TRABECULAR BONE, IN VIVO, FORMATION

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ABSTRACT

Recent evidence indicates that the anabolic response of bone to increased hydraulic pressure, e.g., applied in the medullary canal, is strongly dependent on the pressure gradients of the flow, implying that fluid flow within the matrix is a critical regulatory stimulus to bone mass and morphology (Qin et al., J Biomech, 2003). While the fluid stimulus can be controlled quantitatively and potentially applied for therapeutic in promoting turnover and remodeling, the hypothesis of fluid induced trabecular bone formation was evaluated in an avian ulna model using varied flow rates and magnitudes of fluid stimulus. Thus, bone's response to the specific fluid parameters, i.e., flow rate, pressure magnitude, induced fluid shear stress and cycle number, were examined.

Methods: Total of 12 one-year old male turkeys was used in this study. A special designed fluid loading device with an inside diaphragm was firmly attached on bone via a 4-mm hole near the proximal end of bone allowing oscillatory intramedullary pressure (ImP). On the contralateral ulna, a dummy device was connected into bone with same surgical procedure as sham control. A sinusoidal fluid pressure was applied to the experimental ulna 10 min/day for 4 weeks. Three experimental groups of loading were performed: (A) 30 Hz, 76 mmHg (p-p); (B) 1 Hz, 76 mmHg (p-p); and (C) 30 Hz, 10 mmHg (p-p). To monitor the bone modeling response, all animals were labeled weekly using fluorochrome solutions, e.g., altered by tetracycline and calcein ($15mg\text{-}Kg^{-1}$) through IV. The trabecular regions at both proximal and distal ends were analyzed using histomorphometry measurement.

Results: The results reveal an increase of 22.7%±7.2 in trabecular volume [New Bone Volume / Bone Volume (BV/TV)] ($p<0.05$) for group A (30 Hz, 76mmHg). BV/TV in Group B (1Hz, 76 mmHg) had only 0.5 % increase between loaded and contralateral control bones with no significance. Low magnitude, high frequency fluid stimulation (Group C) increases BV/TV (15.7%±7.4, $p<0.05$).

Discussion: When applying similar physiologic fluid pressure (i.e., 76 mmHg), a higher flow rate (30 Hz) of stimuli generates much higher remodeling response (23% increase) than a lower rate (1 Hz) of loading (0.5% increase). If fluid shear stress is influenced directly by the velocity and the rate of flow, then it's estimated that under similar fluid pressure the loading rate in group A would increase 20-30 times higher than that in the loading group B. Interestingly, those bones loaded at the higher rate (i.e., 30 Hz), even with smaller fluid pressure magnitude (10 mmHg), has shown significant bone turnover (i.e., 16% increase of bone volume). This implies that bone turnover may be more sensitive to the dynamic components of fluid flow than the static pressure alone. These data suggest that high flow rate and associated shear stress, and high cycle number (elevated by frequency) of repetitive flow stimuli indeed have strong influence on adaptive process. Dynamic fluid stimuli may significantly influence fluid perfusion, convection, and surface fluid shear stress, thereby initiating the adaptive response.

PHYSIOLOGIC OSCILLATORY FLUID FLOW IS RESPONSIBLE FOR BONE FORMATION AND INHIBITION OF BONE RESORPTION DEPENDENT ON LOADING MAGNITUDE

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INTRODUCTION: Fluid flow that arises from the functional loading of bone tissue has been proposed to be a critical regulator of skeletal mass and morphology [1]. We have tested this hypothesis by applying oscillatory hydraulic intramedullary pressure (ImP), which has been demonstrated that bone formation can be elucidated by fluid flow stimuli at specific magnitude and frequency of stimuli [2]. Importantly, this bone remodeling response is induced by fluid flow alone in the absence of matrix deformation, which suggest that the motion of intracortical fluid flow, which arises under mechanical loading, has been acted as an important mediator for regulating bone mass and morphology. Although the distribution of loading induced specific fluid parameters, i.e., the magnitude of fluid pressure, pressure gradient, velocity, and fluid shear stress, are not yet clear, bone fluid flow driven by loading may be necessary to explain the adaptive response of bone, which is coupled with pressure magnitude and frequency *per se* [3]. It was demonstrated that intracortical fluid pressure gradient was correlated with applied ImP in a physiological pressure levels. In this study, we have tested dose dependent of the bone adaptive response to a physiological fluid stimulus, driven by low magnitude, high frequency oscillations of intramedullary pressure in an avian ulna model.

METHODS: Under the general isoflurane anesthesia, the left ulnae of 16 adult, one year old male turkeys were functionally isolated via transverse epiphyseal osteotomies [4]. The metaphyses were then covered with stainless steel caps and well sealed with PMMA. Pins inserted transcutaneously through the caps and clamped with a pair of clamps prevented from internal and external mechanical loading. The bone was drilled and tapped and a special designed fluid loading device with an inside diaphragm was firmly attached on bone via a 5-mm in diameter hole near the proximal end allowing ImP oscillation in the marrow cavity. The dynamic fluid pressure was accomplished through a special designed oscillation system, which the fluid pressure can be controlled with varied frequency and magnitude. A sinusoidal fluid pressure was applied to the ulna with the magnitude of 15mmHg (n=4) (close to the marrow pressure generated by animal blood pressure), 76 mmHg (n=4) (close to 700 με peak strain induced marrow pressure) and 105 mmHg (n=4) (pressure induced by bone impact loading) at 30 Hz, 10 min/day, for 4 weeks. One group was left unloading as disuse control (n=4). The adaptive responses of bone were determined through morphometric measurement at the mid-diaphyses; the cortical area of each animal was compared to the control contralateral ulna. The cross sections of mid-diaphyses were cut using a diamond wire saw (Model 3241 Walter EBNER, Germany) resulting approximately 100 μm bone piece. The morphometric images were obtained using microradiograph (HP Faxitron Series). The morphometric change was analyzed by calculating total areal adaptation of periosteal and endosteal surface modeling/remodeling and intracortical porosity, calculating periosteal and endosteal surfaces new bone (NB) formation and intracortical porosity using a custom designed computer software. The ratio of net change of NB and porosity were determined by comparing NB and porosity to the area of original bone.

RESULTS: In the animal group subject to disuse alone, cortex showed a significant decrease in cross sectional area, which is mostly contributed by intracortical resorption or increasing of porosity area, with reduced 6.1±3.0 % compared to the area of contralateral control. While bone loss was primarily through intracortical porosity and secondarily endosteal resorption, no bone resorption was identified at the periosteal surface in any animal. All animals subjected to fluid flow loading showed a maintenance or gain of total bone mass. While NB formation at the endosteal surface showed no significant difference among applied pressures at 15, 76 and 105 mmHg (Fig. 5a), it demonstrated dose sensitivity of NB formation at the periosteal surface. This resulted in an increase of NB as a result of an increase of loading pressure, i.e., 2.4±0.3% at 15 mmHg, 5.0±2.0% at 76 mmHg and 8.4±3.7% at 105 mmHg (Fig. 1). Disuse induced approximately 4.0±1.2% intracortical porosity (Fig. 2). These remodeling experiments have shown nonuniform spatial distribution at endosteal and periosteal surfaces. Interestingly, increasing of ImP magnitude was not necessary to inhibit increase of intracortical porosity; rather it activated remodeling in the cortex (Fig. 2).

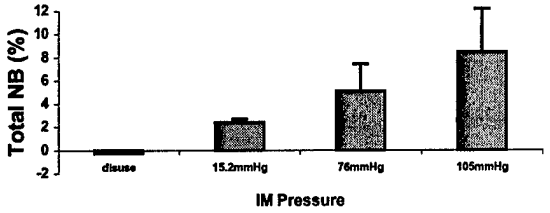

Fig. 1. Histomorphometry of a 100 μm section of the midshaft of ulna analyzed from bones subjected to the disuse control, and ImP stimuli at 15.2, 76.0 and 105.0 mmHg, 10 min per day for 4 weeks. Total new bone calculated by endosteal and periosteal new bone formation, indicated increase as increase of physiological ImP.

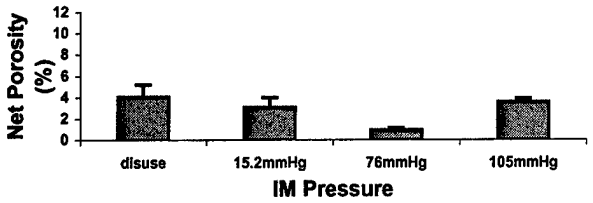

Fig. 2. 4-week disuse resulted in significant intracortical porosity. Fluid flow stimulation inhibited such bone resorption process only at specific pressure magnitude, i.e., 60~80 mmHg.

DISCUSSION: These data confirmed that fluid flow could significantly elucidate adaptive response if applied at physiologic fluid pressure and specific frequency in a short loading duration. The results demonstrated that low magnitudes of ImP can initiate spatial fluid flow in bone and thus stimulate bone adaptive response. At physiological magnitude, ImP can increase and improve the perfusion process through increasing fluid pressure. Within physiological range, new bone formation is proportional to applied fluid flow stimulation. While pressure applied exceeds the physiological intensity or into the pathologic range, it may trigger extensive remodeling process and thus even weaken the quality of bone, i.e., increase of intracortical porosity. In the absence of matrix deformation, the adaptive response was altered by fluid flow loading by means of intramedullary hydraulic pressure. This suggests that oscillation of ImP will influence the perfusion of bone tissue in many ways. For example, IM pressure induced by circulation alone is on the order of 18 mmHg (2.38 kPa), which will provide basic nutritional supply and fluid pressure gradients to the bone. Reducing of this ImP, i.e., aging, bed rest and micro gravity, would influence the fluid perfusion in bone and may substantially stimulate remodeling. However, the mechanism that how cell respond to fluid loading, e.g., via pressure and/or fluid shear stress, is still remained unknown. Nevertheless, these experiments may yield new insights into the mechanisms, at least in the tissue level, by which bone fluid flow initiates and controls bone morphology. If bone is loaded at proper mechanical signal, i.e., magnitude and frequency, it may provide a useful treatment strategy to enhance bone mass.

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